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Comprehensive Kinetic and Structural Studies of Different Flavonoids Inhibiting Diphenolase Activity of Mushroom Tyrosinase[1]

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Abstract—The effect of 4 flavonoids on the diphenolase activity of mushroom tyrosinase was studied using spectroscopic approach. Analysis of kinetic data demonstrated that flavonoids cause a reversible inhibition of the enzyme activity. Further study showed that gallic acid acted as noncompetitive inhibitor, whereas chrysin, naringin and quercetin inhibited the diphenolase activity of mushroom tyrosinase in a competitive fashion. Comparison of the inhibition constants revealed that the strength with which the inhibitors acted on the enzyme activity was ranking as follows: chrysin (K_i 7.90 mM) < quercetin (K_i 7.44 mM) < naringin (K_i 3.04 mM) < gallic acid (K_i 1.5 mM). These data, therefore, suggest that gallic acid is the most potent inhibitor of the enzyme compared to the other flavonoids used.

Keywords: inhibition, kinetics, structure, mushroom tyrosinase, flavonoids
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Tyrosinase (EC.1.14.18.1) is the ubiquitous enzyme involved in the biosynthesis of melanine in a copper-dependent manner [1, 2]. This enzyme catalyzes the hydroxylation reactions leading to the formation of melanins through 3,4-dihydroxyphenylalanine (L-DOPA) and L-DOPA-quinone, using L-Tyr as substrate [3]. Melaninis are engaged in the biosynthesis of hair-color products as well as betalains, the sclerotization of insect cuticle, and defense responses in arthropods, plants, and fungi [4, 5].

From clinical point of view, the inhibition of tyrosinase to treat pigmentation disorders has been an important subject to study [6]. The inhibition of melanogenesis using natural products through blockade of adenylyl cyclase, could be a promise in development of skin remedies and cosmetic products for hyperpigmentation [7]. Hence tyrosinase, one of the signaling molecules involved in the adenylyl cyclase signaling pathway, has recently gained much attention as a potential target for finding agents with the aim of skin depigmentation [3]. It has been previously reported that cupferron, flavonoids, hexylresorcinol, dodecylresorcinol and alkylbenzaldehydes inhibit the enzymatic oxidation of DOPA [8]. Previous studies have revealed that the natural flavonoids, including 5,7-dihydroxyflavone (chrysin), extracted from plants, honey, and propolis, inhibit tyrosinase very effectively in a reversible manner [1, 6]. It has been shown that some flavonoids, the derivatives of benzopyrone, act as copper-chelating inhibitors, inactivating tyrosinase [9, 10]. Additionally, some other flavonoids reported to competitively inhibit L-DOPA oxidation by mushroom tyrosinase (MT) [11]. Despite the fact that flavonoids reversibly inhibit MT [11], the mechanisms of inhibition by which they act on the enzyme activity are yet to be understood. In continuation of our previous researches on MT activity and stability [12–15], in the present work, we carried out comprehensive kinetic and structural studies using spectroscopic methods to understand the mechanisms through which some natural falvonoids, including quercetin, chrysin, naringin, and gallic acid (Fig. 1) inhibit the diphenolase activity of MT activity. Our data clearly demonstrated that the applied flavonoids reversibly inhibit diphenolase activity of MT. Importantly, despite the close structural similarity between the flavonoid derivatives used, these compounds showed differences in their mechanism of inhibition.

MATERIALS AND METHODS

Chemicals. MT, DOPA, Na_2HPO_4, NaH_2PO_4, chrysin, gallic acid, quercetin, and naringin were obtained from Sigma (USA).

[1] The article is published in the original.